Nampt (Visfatin/PBEF) (human) Intracellular ELISA Kit

(Catalog #K4909-100; 100 assays; Store kit at 4°C)

I. Description:

Nampt, nicotinamide phosphoribosy-ltransferase, is the rate limiting enzyme of the mammalian NAD biosynthesis pathway. The circulating NMN (nicotinamide mononucleotide) and NAD are taken up by beta cells and converted to NAD by Nmnat (nicotinamide mononucleotide adenyltransferase) and intracellular Nampt. The Nampt plays a critical role in enhancing life span and protecting against oxidative damage. The Nampt (Visfatin/PBEF) (human) Intracelluar ELISA Kit is to be used for the *in vitro* quantitative determination of intracellular human Nampt (Visfatin/PBEF). This assay is a sandwich ELISA which utilizies a 96-well microtiter plate which was pre-coated with a monoclonal antibody and a purified polyclonal detection antibody. A HRP-conjugated anti-IgG (Detector) and TMB (3,3',5,5'-tetramethylbenzidine) is added to generate a color intensity directly proportional to the concentration of Nampt in the samples. This ELISA is specific for the measurement of natural and recombinant human Nampt. It does not cross-react with human adiponectin, human resistin, human RELM-β, human leptin, human GPX3, human ANGPTL4, human FABP4, human ANGPTL6, human PAI1. The assay range is 0.25 – 16 ng Nampt/ml and a detection limit of 30 pg/ml (based on adding two standard deviations to the mean value of the zero standard).

II. Kit Contents:

Component	100 Assays	Part Number
Pre-coated Microtiter Plate	6x16 well strips	K4909-100-1
Wash Buffer (10X)	2x30 ml	K4909-100-2
Diluent (10X)	2x30 ml	K4909-100-3
Detection Antibody	60 µl	K4909-100-4
Detector (100X)	150 µl	K4909-100-5
Human Nampt Standard (lyophilized, 32 ng)	1 vial	K4909-100-6
TMB	12 ml	K4909-100-7
Stop Solution	12 ml	K4909-100-8
Plate Sealers	2	K4909-100-9
Lysis Buffer (10X)	12 ml	K4909-100-10

III. Storage Conditions:

Reagents must be stored at 2 - 8°C when not in use. Bring reagents to room temperature before use. Do not expose reagents to temperatures greater than 25°C.

IV. Assay Procedure (Read the ENTIRE protocol before proceeding)

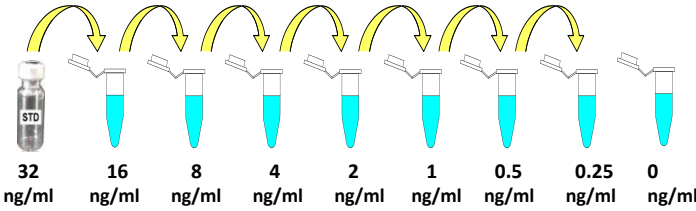
1. Day 1: (We recommend the Samples and Standards be run in duplicate)

a) **Cells Lysates**: Ice-cold 1X Lysis Buffer and 1X Diluent are prepared by 1:9 dilutions with dH2O and placed on ice until needed. Grow cells to 80-90 % confluency. Adherent cells can be scraped off plate and transferred to a tube; suspension cells pipetted to appropriate tube. Centrifuge at 700-1000 x g for 5 min at 4°C and carefully remove and discard supernatant. Wash 1-2 times with ice-cold PBS. Add 200 µl ice-cold 1X Lysis Buffer with 1 mM PMSF (not inluded) per 1 x 10^7 cells and allow to stand on ice for 30 min. Centrifuge at 10K x g for 5 min at 4°C and transfer supernatant to a new tube. The supernatant is the cell lysate and should be freshly prepared and diluted into 1X Diluent. As a starting point 1/10 to 1/1000 dilutions are recommended. If samples fall outside the assay range a lower or higher dilution may be required.

b) **Standards**: Reconstitute Human Nampt Standard with 1 ml of dH_2O to produce a stock solution (32 ng/ml). Mix the Stock solution to ensure complete reconstitution. Allow to sit for a minimum of 15 min. The reconstituted standard should be aliquoted and stored at -20°C.

c) Prepare Standard Curve using 2-fold serial dilutions with 1X Diluent:

To obtain	Add	Into
16 ng/ml	300 µl of Nampt (32 ng/ml)	300 µl of 1X Diluent
8 ng/ml	300 µl of Nampt (16 ng/ml)	300 µl of 1X Diluent
4 ng/ml	300 µl of Nampt (8 ng/ml)	300 µl of 1X Diluent
2 ng/ml	300 µl of Nampt (4 ng/ml)	300 µl of 1X Diluent
1 ng/ml	300 µl of Nampt (2 ng/ml)	300 µl of 1X Diluent
0.5 ng/ml	300 µl of Nampt (1 ng/ml)	300 µl of 1X Diluent
0.25 ng/ml	300 µl of Nampt (0.5 ng/ml)	300 µl of 1X Diluent
0 ng/ml	300 µl of 1X Diluent	Empty tube



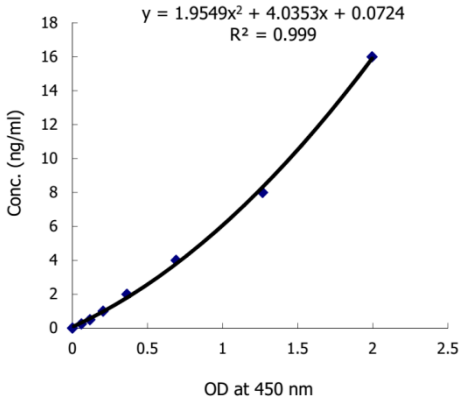
a) Determine the number of 16-well strips needed for the assay and insert them into the frame for current use. The extra strips should be resealed in the foil pouch and can be stored at 4°C for up to 1 month.
b) Add 100 µl of the Standards and Samples into the appropriate wells in duplicate.
c) Cover the plate with plate sealer and incubate at 4°C overnight.

2. Day 2: (Note: the Detector must be used within 1 hr of preparation)

a) Prepare 1X Wash Buffer: Dilute 10X Wash Buffer 1:9 with dH_2O.
b) Warm Detection Antibody to room temperature. Dilute the antibody 1:250 in 1X Diluent (8 µl antibody + 1992 µl 1X Diluent). Diluted antibody cannot be stored.
c) Remove plate from 4°C, aspirate and wash 3 times with 300 µl of 1X Wash Buffer.
d) After last wash, tap inverted plate on a stack of paper towels. Complete removal of liquid is essential for good performance.
e) Add 100 µl of Detection Antibody to each well.
f) Cover plate with plate sealer and incubate for 1 hr at 37°C.
g) After about 30-45 min prepare 1X Detector: Dilute 100X Detector 1:99 with 1X Diluent (100 µl Detector + 9.9 ml of 1X Diluent).
h) Remove plate from 37°C, aspirate and wash 3 times with 300 µl of 1X Wash Buffer.
i) After last wash, tap inverted plate on a stack of paper towels. Complete removal of liquid is essential for good performance.
j) Add 100 µl of 1X Detector to each well.
k) Cover plate with plate sealer and incubate for 1 hr at 37°C.
l) Warm the TMB Solution and Stop Solution to room temperature.
m) Remove plate from 37°C, aspirate and wash 5 times with 300 µl of 1X Wash Buffer.
n) After last wash, tap inverted plate on a stack of paper towels. Complete removal of liquid is essential for good performance.
o) Add 100 µl of TMB Solution to each well.
p) Allow the color to develop at room temperature in the dark for 10 min.
q) Stop the reaction by adding 100 µl of Stop Solution to each well.
r) Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue product that turns yellow when Stop Solution is added.
Caution: Stop Solution is Corrosive
s) Measure the OD at 450 nm in an ELISA plate reader within 30 min.

3. Calculations:

a) Average the duplicate readings for each standard and Test Sample.
b) Subtract the average 0 ng/ml standard from each of the above.
c) Generate a Standard Curve by plotting the average absorbances on the horizontal (X) axis vs. the corresponding concentration (ng/ml) on the vertical (Y) axis. (See Typical Data below)
d) Calculate the Test Sample Nampt concentrations by interpolation of the Standard Curve regression curve as shown above in the form of a quadratic equation.
e) If the Test Samples were diluted, multiply the interpolated values by the dilution factor to calculate the corrected human Nampt serum concentrations.



VI. Performance Characteristics:

1. Intra-assay Precision: (2) samples of known concentration were assayed in replicates (10) times to test precision within an assay.

Samples	Mean (ng/ml)	SD	CV (%)	n
# 1	244.46	6.66	2.73	10
# 2	248.13	24.21	9.76	10

2. Inter-assay Precision: (2 samples of known concentration were assayed in (6) separate assays to test precision between assays.

Samples	Mean (ng/ml)	SD	CV (%)	n
# 1	209.59	8.42	4.02	6
# 2	251.04	18.58	7.40	6

Note: Mouse Nampt shows weak cross-reactivity in this assay (5 %).
Rat Nampt shows weak cross-reactivity in this assay (15 %).

3. Recovery: Human cell lysates were spiked with known concentrations of human Nampt. The recovery averages were 98 % (range from 90 % to 105 %

Samples	Average Recovery (%)	Range (%)
# 1	96.36	95 - 105
# 2	102.62	95 - 105

Technical Hints and Limitations:

- It is recommended that all standards and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100 µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
- Crystals could appear in the 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
- Once reagents have been added to the 16-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep TMB Substrate Solution protected from light.
- The Stop Solution consists of Sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

Troubleshooting:

PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be followed as indicated in the manual.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of detector too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.

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